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**A Non-lethal, Repeated Testing, Anesthetized Canine Model
for the Evaluation of Effectiveness of New Forms of
Prophylaxis and Therapy for Cyanide Intoxication**

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ABSTRACT

Acute cyanide intoxication has most often been modeled through the bolus intravenous administration of a lethal amount of sodium or potassium cyanide which provides reproducible effects and represents the most severe challenge to any new form of prophylaxis and therapy. Inhalation of cyanide leads to a similar acute onset of toxic signs which is controlled by the rate and depth of respiration. The cyanide induced halt in respiration also halts the continued absorption of cyanide leading to a well defined, consistent end point of the amount of cyanide absorbed. Regardless of the abundance of cyanide in the ambient air, the casualty can only absorb cyanide during respiration. A slow intravenous infusion of cyanide which is continued only until respiratory arrest is achieved should define the same limit of cyanide intoxication.

Cyanide intoxication defined by the amount of sodium cyanide infused to induce respiratory arrest (RA) in pentobarbital anesthetized dogs provides the basis for the development of a useful repeated testing animal model. Utilization of the RA yields a surrogate endpoint in the anesthetized dog model and provides a non-traumatic, reproducible procedure to estimate the lethal level of CN in each dog as well defining the protective effect of pretreatments and antidotes.

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INTRODUCTION

Acute cyanide intoxication leads to a momentary increase in the rate and amplitude of respiration followed by a very sudden halt in respiration leading to death of the untreated casualty. Inhalation of cyanide leads to a similar acute onset of toxic signs which is controlled by the rate and depth of respiration. The cyanide induced halt in respiration also halts the continued absorption of cyanide leading to a well defined, consistent end point of the amount of cyanide absorbed.

During the period of cyanide infusion the total blood-cyanide levels increase to a maximum and plateau following respiratory arrest. The amount of cyanide which must be infused to achieve respiratory arrest is very reproducible in repeat administration of cyanide to the same animal and also between animals. The amount of iv infused cyanide required to induce respiratory arrest in animals which have been protected with sufficient sodium nitrite to develop 3% of methemoglobin is increased by a reproducible factor.

The system developed in the current study of cyanide intoxication utilizes a slower intravenous infusion of cyanide which is halted as soon as respiration stops provides a reproducible, well defined endpoint of cyanide intoxication which occurs several minutes before the animal would succumb to cardiovascular collapse. In this model cyanide induced lethality is defined as the respiratory arrest dose. Treatment of cyanide intoxication can be accomplished by the i. v. or i.m. route once the respiratory arrest dose has been achieved and the animal can survive to be tested again with the same or similar formulation. The multiple comparison possibility of this preparation leads to a continuous comparison of various forms of therapy in the same animal.

METHODS

Adult beagle dogs (9-12 Kg) anesthetized with pentobarbital (32 mg/kg i.v.) were intubated and fitted with a pneumotachometer. I.V. catheters (veno-cath) were placed in the jugular vein for the collection of blood samples and in the saphenous vein for the infusion of cyanide solutions. Blood samples were monitored for total cyanide levels and methemoglobin activity (Radiometer, OSM-3 modified for canine blood).

ACUTE cyanide intoxication utilized a bolus i.v. injection of 2.5 mg/kg NaCN followed by therapy 2.0 min after initiation of the i.v. injection.

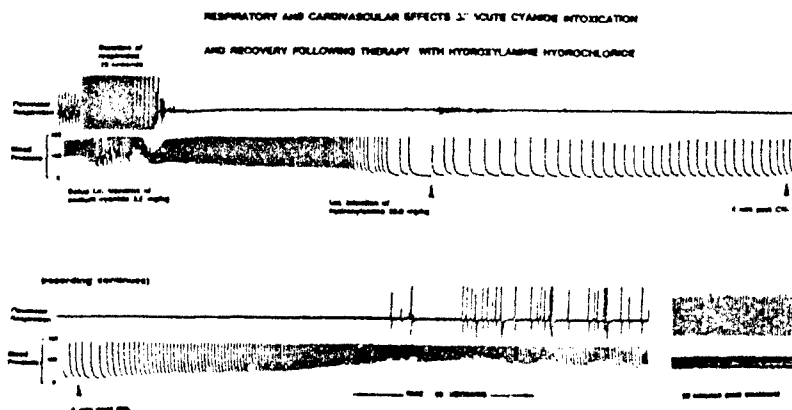
INTRAVENOUS INFUSION of cyanide was carried out at a rate of 7.0 mg/min until respiratory arrest occurred and therapy was applied 0.5 minutes after the onset of respiratory arrest.

Therapy consisted of an intramuscular injection of HA (Hydroxylamine Hydrochloride) (20 mg/kg) or DMAP (Dimethyaminophenol) (2.0 mg/kg). Pretreatment consisted of an i.m. injection of 5 mg/kg Sodium Nitrite administered 30 minutes before exposure to sodium cyanide. The duration of respiration during the infusion of cyanide was monitored and calculated from a physiological recorder.

RESULTS

A. ACUTE BOLUS INJECTION cyanide intoxication model

Fig 1 Bolus intravenous injection of 2.5 mg/kg NaCN in an anesthetized dog followed by therapy with hydroxylamine illustrates respiratory arrest and recovery with a methemoglobin forming compound



B. INTRAVENOUS INFUSION cyanide intoxication model

Fig 2 control intravenous infusion of 7 mg/min NaCN in an anesthetized dog until respiratory arrest occurs; demonstrates a lack of recovery of respiration without therapy.

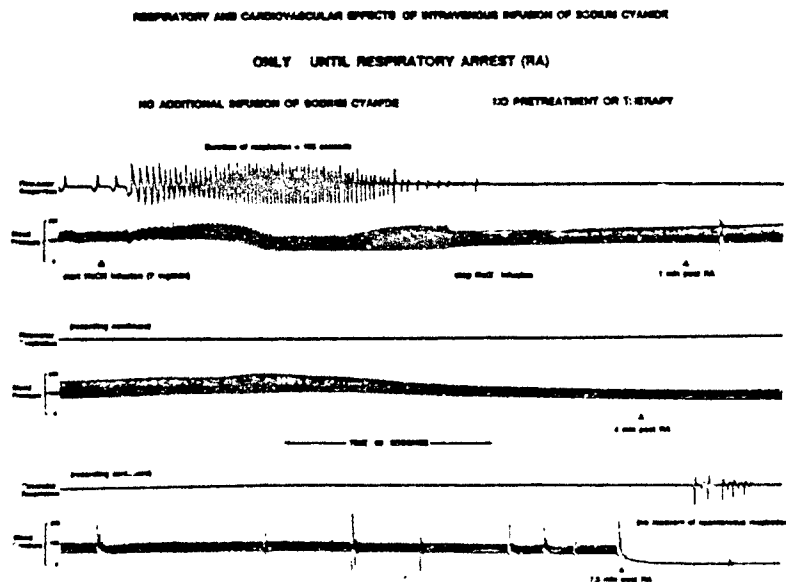
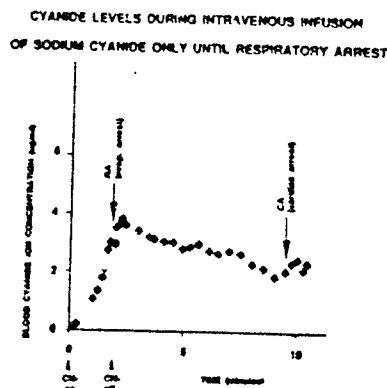


Fig 3 Corresponding blood cyanide levels in the same animal



C. NON-INVASIVE INTRAVENOUS INFUSION cyanide intoxication model

Fig 4 (upper) Duration of respiration in control anesthetized dog during infusion of NaCN,

(lower) Duration of respiration in the same anesthetized dog pretreated with 5 mg/kg Sodium Nitrite

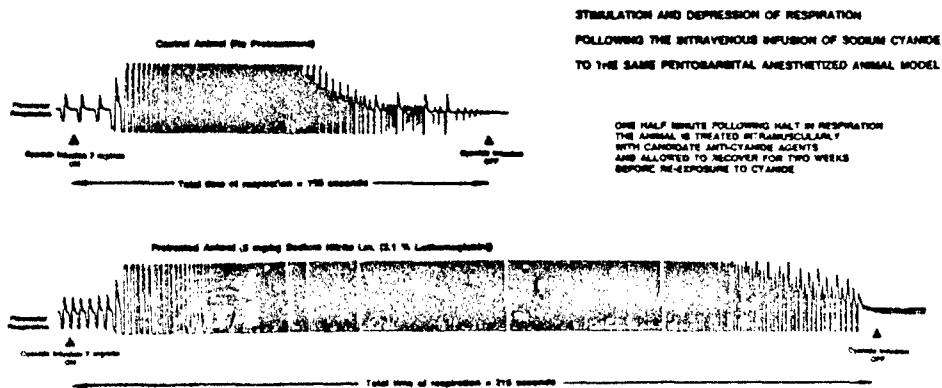


Fig 5 Corresponding blood cyanide levels in the same dog demonstrate the increased total blood level of Cyanide during methemoglobin (induced by sodium nitrite) pretreatment

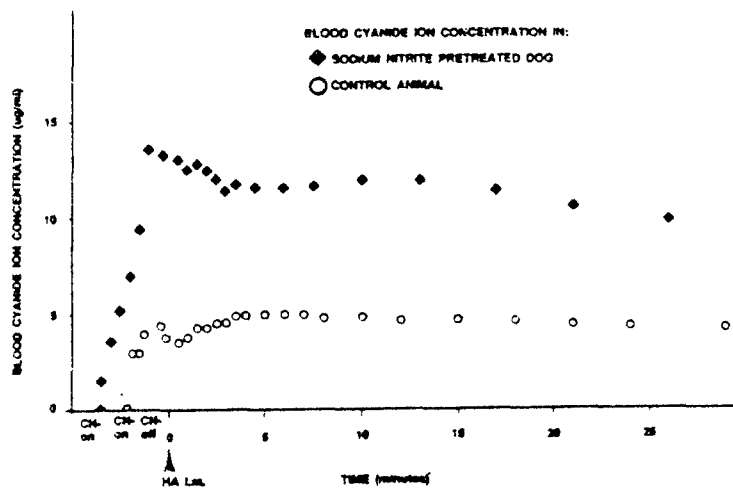
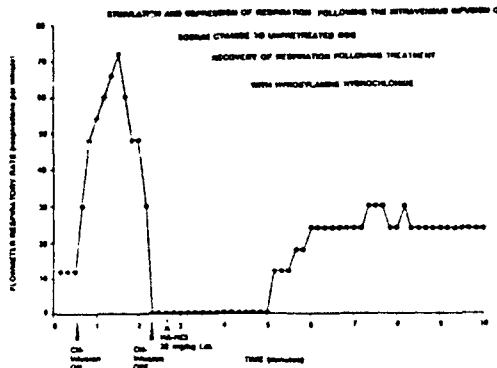


Fig 6 a. duration of respiration in control anesthetized dog during the intravenous infusion of NaCN, respiratory arrest and recovery following therapy with hydroxylamine



b. duration of respiration in the same anesthetized dog pretreated with sodium nitrite during the intravenous infusion of NaCN leading to respiratory arrest and recovery following therapy with hydroxylamine

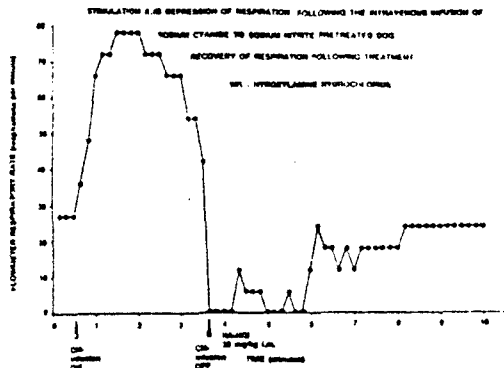


Fig 7 Reproducibility of the amount of NaCN required to induce respiratory arrest in control and methemoglobin (induced by sodium nitrite) pretreated anesthetized dogs.

SUMMARY OF NaCN INFUSION DATA

RESPIRATORY ARREST DOSE OF CN:

IN CONTROL ANIMALS =
 1.21 ± 0.06 mg/kg (n = 12)

IN PRETREATED ANIMALS =
 2.45 ± 0.37 mg/kg (n = 8)

PROTECTIVE RATIO induced by 5 mg/kg i.m. NaNO_2

RA pretreated/ RA control = PR

average within each animal
 $= 2.01 \pm 0.32$ (n = 6)

average among animals
 $= 2.45 / 1.21 = 2.02$

DISCUSSION

Respiratory arrest (RA) following intravenous infusion of cyanide provides a definite end point to stop agent infusion. The RA endpoint is a surrogate endpoint for a known lethal event. The RA endpoint can be used to initiate and evaluate the effectiveness of candidate antidotes.

The sodium cyanide infusion has been carried out repeatedly in the same animal leading to a consistent respiratory arrest (RA) response and a consistent recovery of the animal following therapy with a methemoglobin forming compound. The RA response is consistent within each animal and between animals.

The model provides a non-lethal, non-traumatic approach in an anesthetized canine model to approximate a normally lethal event. The nembutal anesthetized animals do not endure convulsions and recovery from anesthesia is not delayed as a result of the exposure to cyanide.

Treatment of Cyanide intoxication required only the temporary formation of methemoglobin with hydroxylamine or dimethylaminophenol, no additional therapy with sodium thiosulfate was required to ensure complete recovery.

The model demonstrates the effectiveness of methemoglobin pretreatment by allowing the animal to breathe longer and to absorb greater levels of cyanide before respiratory arrest occurred.

The same rate of recovery of the animal occurred following therapy when the animal was pretreated with a methemoglobin forming compound.

CONCLUSION

A non-traumatic, non-lethal, repeated testing, anesthetized canine model has been developed to approximate cyanide toxicity and to evaluate the effectiveness of systems of prophylaxis and therapy.